MOUNTING APHIDS AND OTHER SMALL INSECTS ON MICROSCOPIC SLIDES[1]

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In order to systematically study many small insects such as aphids, thrips, and coccids, it is necessary to prepare the specimens so that they may be critically examined under high magnifications. Although unmounted or temporarily mounted specimens may be superficially investigated by means of a hand lens or a binocular microscope, a properly prepared and mounted specimen is required for the careful examination with a compound microscope necessary for exact determination of species and for morphological study. From earliest times, in the examination of these small insects, it has been a common practice to mount them directly into any available medium on a glass slide and covered with a very thin glass cover slip. Specimens so mounted may be preserved almost indefinitely against the inroads of time, moisture, corrosion, fungi, and predacious insects that so often destroy pinned and otherwise exposed insects.

Since 1909 I have been studying plant lice of the family Aphididae and have had occasion to examine many methods of preparing these rather delicate, soft-bodied insects for study both in America and in Europe and have also examined much mounted material received from systematists from all parts of the world. I do not know who first perfected the technique of the Canada balsam mount, but the earliest slides of aphids that I have examined were those prepared by Francis Walker which are in the British Museum of Natural History and are now approximately 100 years old. Although the balsam has darkened greatly, the specimens are still in fair condition for study with a compound microscope. Other early students of aphids mounted them on pins or on points and consequently most of the specimens of species described before Walker's time have been lost. Thus the innumerable species described by Linnaeus, Fabricius, Kaltenbach, Schrank, Koch, Geoffroy, De

[1]These recommendations and procedures are also applicable to Acarina and to other small insects, including the Apterygota, Coccidae, Anoplura, Mallophaga, Thysanoptera, etc.

Geer, and other contemporaries in Europe and those of Harris, Fitch, Walsh, Rafinesque, Haldeman, Ashmead, and others in America, have mostly disappeared and there are no specimens such as types or cotypes left to guide their successors.

During recent years, most systematists have mounted aphids in the accepted manner on glass slides directly into balsam or euparal. Certain more exacting and careful workers have also cleared the specimens by cold or hot treatment in a water solution of KOH or NaOH followed by staining and preparatory to mounting in media.

Aphids are somewhat more difficult to clear than most other small insects because of the embryos which should be removed from the bodies of the females before a satisfactory transparent and stained mount can be secured. The embryos, if not removed, may completely fill the body cavity and obscure many of the important details necessary for the correct determination of the species. Staining of the embryos within the bodies results in an opaque mass of little use to the systematist. Staining may also obscure much of the pigmentation so useful in classification.

In order to improve the technique in the preparation of more satisfactory and permanent mounted specimens of aphids, a careful study was begun at the British Museum of Natural History, London, in the fall of 1936, and from there continued in Belgium, Holland, Switzerland, Austria, Germany, France, and the United States up to the present time. The results of the studies are also based upon the experience of many aphidologists.

I. Preservation of Specimens for Permanence or for Subsequent Mounting

1. Living or freshly collected specimens

The most easily prepared and satisfactory mounts are from freshly collected living specimens. They may be removed from the host plants and killed in 95 per cent ethyl alcohol. This solution also thoroughly wets the bodies and wings and prevents the latter from sticking together, becoming misshapen, and from collecting air bubbles. They are then transferred immediately to the clearing solution.

2. Specimens preserved in liquids

(1) *In alcohol.* A solution of 70 to 95 per cent ethyl alcohol is very satisfactory for temporary or permanent preservation of

aphids. Specimens remaining in such concentrations for twenty or more years may be satisfactorily cleared for mounting.

(2) *In formalin.* Specimens preserved for many years in this fluid become hardened and are very much more difficult to clear and mount than those preserved in alcohol. If first soaked for 48 hours or more or heated in water, they may yield satisfactory specimens. Formalin is not recommended as a preservative for aphids.

(3) *In lactic acid.* A solution made up of 35 per cent lactic acid, 50 per cent alcohol, and 15 per cent water is a very satisfactory preservative for a short period of time, not much over a month. This preservative does act as a clearing medium but even so the embryos should be removed before mounting if the specimens are to be stained. A longer period in the preservative may cause the specimens to distintegrate and to become very difficult or impossible to mount.

3. Dried specimens

Aphids that have been dried may be treated in the same way as alcoholic or fresh specimens. Aphids preserved on herbarium specimens may be recovered if care is taken to remove the fragile insects in their entirety. Thus dried bodies on herbarium specimens preserved dry for over 100 years were successfully removed and mounted. Frequently bottled alcohol specimens dry up because of leakage around stoppers. Such specimens may be treated in the regular manner and usually clear up perfectly, seldom requiring the removal of body contents.

II. Clearing Specimens Preparatory to Permanent Mounting on Glass Microscopic Slides

There have been many methods proposed and used for mounting aphids on slides. The experience, technique, and objective of an aphid specialist has a great deal to do with the results obtained. Since specially prepared specimens are so much better for study than those simply dropped into a mounting medium, it is unnecessary to repeat the simple methods of the past.

Caustic Treatment (KOH or NaOH). Many aphidologists prefer to use these alkali chemicals for clearing aphids. It is the usual practice to make up a stock solution of 5 or 10 per cent for use as needed. It is now possible to procure gram pellets which are much more satisfactory, as small quantities may be readily prepared by adding a pellet or two as needed. All the difficulties occasioned by stock bottles of these solutions are eliminated.

There seems to be a growing preference for NaOH because it does not appear to be so destructive as KOH to the pigments and to the integument of these fragile insects. Both of these chemicals are also very destructive to the delicate wings of aphids and often render them unfit for mounting and study.

1. *Cold Solution.* The specimens are placed in a small container; vial, watch crystal or slender dish—completely immersed in a solution of the caustic clearing agent and put aside until the desired transparency is secured. From a few days to a week or even more time may be required. Specimens so treated frequently become very pale or completely transparent and even destroyed if left too long.

2. *Hot Solution.* By heating or boiling the specimens in the caustic solution the process of clearing may be reduced to a few seconds or minutes. Boiling is often quite injurious to the specimens unless carefully done. For aphids it is not to be recommended except for preserved species belonging to the dark forms of such genera as *Astegopteryx*, *Thoracaphis* and *Aleurodaphis.*

3. *Washing and staining.*

(1). Removing body contents. When the specimens are sufficiently cleared the embryos may be removed by using a suitable teasing instrument (Fig. 1) constructed by heating, flattening, and shaping a large steel needle point. Because of the delicacy of the treated tissues of the specimens it is often difficult to squeeze and tease out the embryos because the bodies collapse and prevent the "bellows action" that may operate to free the embryos from the body. Males and immature females usually clear without the necessity of removing the body contents.

In clearing, the wings often swell up like bags and are so fragile that great care must be exercised to deflate them without destroying them completely.

(2). Staining. After clearing, the specimens are removed to a clean container with water, acetic acid, or a suitable stain. Ordinarily, basic fuchsin or magenta is satisfactory, but fast green or other stains may be employed. NaOH-fuchsin may also be used, whereby staining and clearing may be accomplished at the same time. This step is followed by removal of the specimens to glacial acetic acid.

(3). Dehydrating. The third step is to remove any water and excess acid and stain by transferring to 95 per cent alcohol.

(4). Destaining. If the specimens are stained too densely the excess color may often be removed by transferring to water. Care is taken to remove just the right amount of stain. The aphids are then returned to alcohol. Ordinarily 95 per cent alcohol is adequate for satisfactory dehydration.

(5). Fixing and Clearing. The brilliance of the specimens is often improved by transferring them from the alcohol to clove oil, xylol, or a similar reagent. Ordinarily one may simply add a small quantity of clove oil to the specimens already in alcohol. Small globules of fat or soapy material may also be removed by the addition of a small amount of xylol.

Chloral Hydrate, Alcohol, Lactic Acid, Carbolic Acid, and Water Clearing Mixture

A solution of these components appears to have originated among aphidologists in Holland and has come to be generally used. The formula and procedure are as follows:

(1) Place preserved or live specimens in 70 per cent alcohol and heat over a boiling water bath for 10 to 15 minutes.

(2). Transfer to a solution of 70 per cent lactic acid and similarly heat for 15 to 20 minutes. (Small pale whitish or yellowish species for only 10 minutes).

(3). Transfer to a mixture of a saturated solution of chloral hydrate, to which a small amount of carbolic acid crystals are added, and heat over a hot water bath for only a few minutes or until the color is greatly lessened or disappears.

(4). Remove the body contents—especially the embryos.

(5). Mount directly into Berlese mixture, a modification of De Fauer's Fluid, or into a similar medium on the slide without washing or further treatment.

(6). Heat lightly for 10 or 15 minutes to relax the specimens but not to give rise to bubbles or air pockets under the cover slip.

(7). After a week or more, clean slide and ring with suitable ringing compound discussed further on.

Chloral hydrate and lactic acid both have a tendency to destroy aphid tissues very slowly and for that reason it is necessary to remove these chemicals by washing thoroughly and dehydrating in 95% alcohol followed by clearing in clove oil or similar oil *before mounting them in a more permanent medium such as balsam, euparal and dammar.*

LACTIC ACID, ALCOHOL, CARBOLIC ACID, AND WATER CLEARING MIXTURE

(1). *Formulation.* By far the simplest and most satisfactory solution for clearing aphids is one prepared as follows:

Stock Solution

Lactic acid	45 parts
Acetic acid	5 parts
Ethyl alcohol	30 parts
Water saturated solution of phenol	5 parts
Water	15 parts

This formula may be modified somewhat by adding more lactic acid or water to specimens long preserved in 80-95 per cent alcohol.

(2). *Heating.* Small stender dishes, 1¼ inches in diameter, are very suitable containers for handling the aphids during the clearing, heating and staining processes. A good procedure is to empty the aphids from the preservative directly into the dishes, draw off excess alcohol with a pipette and add the clearing solution. Appropriate labels may be attached to the covers. The covered dishes are then put into petri dishes and placed in a constant temperature oven and maintained at a temperature of approximately 120° F. or 49° C.

Freshly collected specimens may be heated sufficiently in one hour, whereas alcoholic specimens of long standing require from 24 to 48 hours or even longer. There is considerable difference in the relaxing and clearing of different species of aphids by this process. The most difficult specimens to clear are certain dark species of the genus *Aphis* which have long been in alcohol. The proper amount of heating may be determined by the clear appearance of the bodies and by actual testing of a few individuals. It may often be necessary to return inadequately cleared specimens to the oven for further heating. Injury by overheating has not been noted.

In developing this process the cleared specimens are removed from the lactic acid solution, the body contents and especially its embryos removed, transferred to 95 per cent alcohol, then to clove oil and finally mounted in the desired medium.

Dried specimens, alcoholic females devoid of embryos, and males, require no other treatment, but females which are full of embryos or eggs require special attention to remove these from

the bodies. To do this the aphids taken from the oven are transferred to the more accessible syracuse watch crystals and the embryos or eggs removed by special "teasers" under a binocular microscope. These teasers (Fig. 1) are of various sizes designed to just cover the entire body of the victim to be operated upon. Pressure from above disrupts the body wall at different points—usually along the sides—and the embryos are forced out by a carefully directed up and down bellows-like motion with just enough pressure to remove the embryos and other body contents without injuring or removing the legs, antennae, and wings. It is not always possible to secure absolutely perfect specimens from poorly preserved material and from fragile or very delicate species, but with care and patience very perfect and beautiful mounts may be secured. Freshly collected specimens yield the most perfect mounts.

When spread, these deflated skins do not assume the exact body contours of the living specimens but they permit the most discriminate and painstaking study of all the body characters that are important in determining the identity of the species. The pigmentation, sculpturing, vestiture, and other characteristics are preserved perfectly and can be reproduced by drawings and by microphotography.

Cleared specimens appear to vary considerably as to their rigidity following the various steps leading to their final immersion in the mounting medium. If not properly dehydrated and hardened during the last step, the antennae and legs often collapse when transferred to the mounting medium. This shrinking is caused by the sudden difference in the osmotic pressure between the lighter body impregnating fluids and the heavier mounting medium. Clove oil, xylol, and similar fixing solutions harden the tissues so that this collapse may be lessened or prevented. Apparently the effects of the preservative may be responsible for a hardening or softening of the body and appendages. Or perhaps the fact that newly molted specimens had not sufficiently hardened before they were collected and treated may result in their collapse more readily than the older and more mature individuals.

III. Mounting—General

Insect collections should be prepared for permanence. It has already been noted that the ordinary methods of pin and point mounting of such small and fragile insects as aphids are very temporary and useless for collection and museum purposes.

Slide mounts are not always satisfactory over a long period of time unless careful preparation and the most permanent types of mounting media are employed. At the present time there is no way of knowing what particular kinds and combinations of chemicals will prove the most satisfactory for these purposes over a period of hundreds of years. Perhaps we cannot expect such enduring qualities of a medium that must be so easily manipulated and perfectly adapted to all the requirements of a satisfactory mount that may be subjected to study by a high-powered microscope or the even higher magnification of an electron microscope.

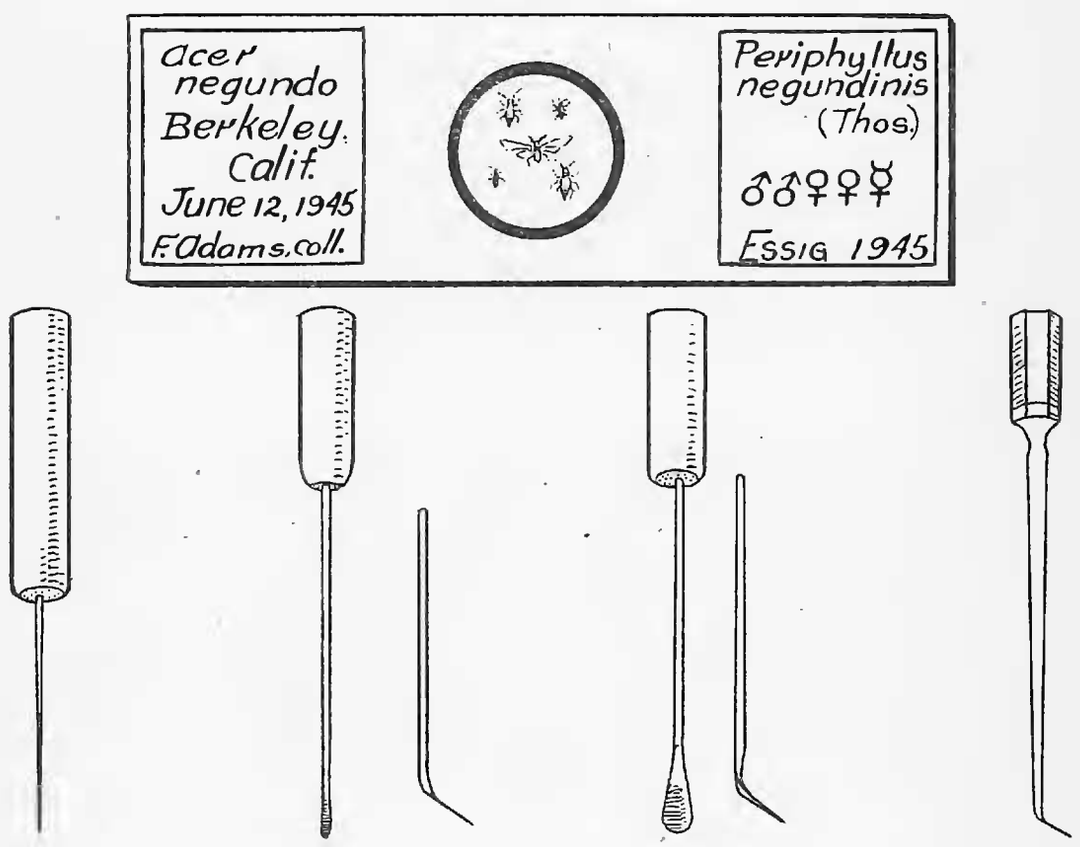

Fig. 1. *Top.* Glass microscope slide showing arrangement of aphid specimens, the ringed cover slip and suitable method of labeling. *Bottom.* Needle point and angled "teasers" used for the removal of the embryos and other body contents of the aphids. A large assortment of sizes may be desirable. The handles may be as long as needed. Natural size. (Drawing by Frieda Abernathy.)

(1). Canada Balsam has long been the most versatile mounting medium for small insects. Its important qualities are: (1) ease of manipulation; (2) ability to give up bubbles and air pockets; (3) refractive qualities; its index of refraction being 1.535;

(4) stability and durability; and (5) cheapness and availability. Some of the objectionable qualities are: (1) brittleness and tendency to dry out and crystallize over a period of years: this condition may often be temporarily corrected by adding xylol to the mount; (2) tendency to discoloration. Clear white balsam mounts prepared 20 to 30 years ago have become darker around the periphery under the cover glass. This darkening is gradually extending inwardly—a condition which might have been prevented by ringing with a suitable ringing compound. Unfortunately such of the old ringing materials as Brunswick black and white lead compounds proved to be quite unsatisfactory for such purposes.

(2). Euparal. This is a synthetic mounting medium composed of camsal, eucalyptol, pyraldehyde, and sandarac, which was first made available in the United States about 1927. It was accepted with some hesitation and much misgiving by most entomologists. Its cost was about eight times that of Canada balsam and this prohibited its extensive use in teaching and general laboratory work. Its durability has not yet been proven by actual use over a long period of time. However, it was soon adopted by many discriminating biologists and at the outbreak of the last great war in 1942, it had become a stable laboratory essential. Its importation from England and from Germany, where it was commercially formulated, was cut off during the war and the supply completely gave out in most places. Just now it is being sent over to this country from England and is being offered at the announced price of 23 dollars per pound. Only small deliveries have been forthcoming. As a substitute I have been using a local "Wherle" Euparal mixture which appears so far to be quite satisfactory. It has a tendency to darken somewhat or turn greenish in bulk, but the thin layer under a cover slip has remained colorless and clear over a period of four years. It has a refractive index of 1.483. Euparal is also marketed under the name Diaphane at $7.50 per pound.

Specimens may be transferred into Euparal directly from 95% alcohol or from a mixture of 95% alcohol with a small amount of xylol, oil of cloves, oil or Bergamot or other clearing agent. Like Canada balsam, Euparal readily gives up air bubbles. It sets quickly and apparently does not crystallize.

(3). Berlese Mounting Medium (A Modification of De Fauer's Fluid). As now formulated, this appears to be a satisfactory mounting solution. There are many modifications and formulations in use and all of them apparently give satisfactory

results for those who have devised them. A good formula is as follows:

Distilled water	20 cc.
Chloral hydrate	160 gr.
Gum arabic	15 gr.
Glucose syrup	10 cc.
Acetic acid	5 cc.

These ingredients may be formulated in the order listed over a hot water bath or in an oven at 50° C or 123° F. The resulting fluid should be filtered through a Buchner funnel and suction pump, or through Whatman No. 5 filter paper, or clarified in a centrifuge. It has a refraction index of about 1.461.

Specimens of aphids may be taken directly from water, alcohol or 10% acetic acid in distilled water. It is also possible, and often advisable, to mount living specimens directly into the medium after wetting them in alcohol to eliminate air bubbles.

After mounting, the slides may be heated for a short time over a hot plate or in an oven at about 120° F. This treatment relaxes and somewhat expands the specimens and promotes rapid clearing. It also seals the cover glass. Care must be taken to prevent the formation of bubbles.

After a period of two to four weeks the slide preparation should be ringed with a suitable ringing compound, as listed further on.

(4). Dammar. This is a resin derived from Australian conifers formerly of the genus *Dammara* (now *Agathis*). Refined to a colorless mounting medium, it has the excellent qualities of balsam and Euparal for which it is a satisfactory substitute. It has an index of 1.520 and appears to remain perfectly clear under the cover slip although it may cloud some in bulk. Specimens are prepared as for mounting in balsam or Euparal.

(5). Polyvinyl Alcohol. This is a relatively new mounting medium recently developed. The methods of formulation vary considerably which indicates that exact proportions of the various ingredients are not too important.

The recommendations for preparing the mounting medium with polyvinyl alcohol grade RH-349-N by the manufacturers, E. I. DuPont de Nemours & Company, Inc., are as follows:

A.—PVA Stock Solution:

To 80 cc of distilled water add PVA in small amounts until the mixture has the consistency of a thick syrup and attains a

volume of approximately 100 cc. Heat in a water or steam bath until homogeneous and milky in appearance. Allow to cool. Reheat to produce a clear mixture. Cool. Strain through fine copper gauze. (Some investigators recommend filtering the hot solution through 4 or 5 layers of filter paper.)

B.—PVA Stock Mounting Medium:

PVA Stock solution	56 cc.
Phenol crystals	22 grms.
Lactic acid (C.P.)	22 cc.
Picric acid (for staining)	1 grain.

Mount specimens into PVA directly from 95 per cent alcohol or from water in the regular manner, being careful to eliminate as many bubbles as possible. This will require some practice and experience. Fresh or preserved aphids may be wet in alcohol and then immersed in water and mounted directly into PVA without clearing or removing the embryos or body contents, but these specimens are not nearly as satisfactory for microscopic study as those properly cleared and stained.

After several days or weeks the excess PVA may be removed with a damp cloth and the slides ringed in the ordinary manner.

(6). Clarite and similar synthetic compounds:

The new plastic material, Clarite, makes a very good mounting medium for aphids and other insects. The specimens, after clearing in a proper solution and dehydrating in alcohol, may be transferred to xylol, toluene, or clove oil and thence directly into clarite. It is very important to have just the right consistency of the medium for eliminating bubbles. A 60 per cent solution of clarite in toluene, by weight, appears to give the most satisfactory results for aphids. The solution may be thinned with toluene, 1° C or 2° C grades. It is nearly water white and has a refractive index of 1.544 and a melting point of 145° to 150° C.

IV. Cover Slips

Circular cover slips are preferable to squares because they can be ringed with a cement that protects the mounting medium from desiccation, discoloration and possible crystallization. If temporary mounts only are desired, there is no need to go to the additional trouble of attempting to use permanent mounting media or for ringing the slides. But for collections, and especially for

types of all kinds, it is most desirable to produce as permanent mounts as possible. For such mounts, ringing the cover slips to seal the mounting medium may be an important factor. (Fig. 1.) The ringing may be repeated from time to time, every 10 or 20 years if required.

V. Ringing Compounds

Slide ringing has been practiced for many years by botanists, plant pathologists, nematologists, and a few entomologists. Brunswick Black or alphabet varnish, Japan gold size, zinc white, and other materials were used, but without very satisfactory results.

Materials that are now available and very much better for ringing are:

(1). *Murrayite.* An English product used mostly for sealing jars and vials containing specimens preserved in alcohol. It is a clear light brown compound soluble in xylol and similar solvents that is easily applied, quick-drying, and thoroughly satisfactory for ringing slides.

(2). *Thorne ringing compound.* This new material was evolved by Gerald Thorne, nematologist, Bureau of Plant Industry, U. S. Department of Agriculture, in 1935 for ringing cover slips for nematodes mounted in a glycerine medium. The formulation is:

Nitrocellulose solution	2 parts
A. D. M-100 (a polymerized linseed oil product used in the paint trade)	1 part
Thinners—Butyl acetate or acetone	

The material is transparent and may be colored to suit the user. A small amount of an oil soluble red pigment is used by the originator and others. Its sale is at present somewhat restricted but it will no doubt be made readily available in the near future. It is a very valuable addition to ringing compounds and is apparently suitable for many purposes. It is very satisfactory and quite inexpensive.

VI. Labels and Labeling

The proper mounting of small and minute insects on glass microscopic slides is one of the most permanent methods for the preservation of insects. Therefore, great care should be taken to insure equal permanence of the essential data that should accompany the specimens.

This data may be scratched on by a diamond, but the results are not altogether satisfactory because it is difficult to scratch the letters distinctly and the finished inscriptions are trying to read.

Gummed paper labels prepared commercially are generally used. Unfortunately in most labels neither the paper nor the adhesive have lasting qualities. Labels of slides prepared ten or twenty years ago are now in bad shape. They may have so deteriorated as to be either falling to pieces or so discolored as to be illegible. The adhesive may also have disintegrated. The ink often used was frequently not permanent and may have faded. The labels of slides prepared in 1909-1920, and even later, have recently been specially treated to prevent further disintegration. Most labels are carelessly and poorly applied with only portions properly and entirely stuck to the glass slides. Handling has caused the edges to roll up and tear off and eventually the writing has become so defaced as to be unsightly and illegible.

For permanency, slide labels should be made of thin linen or rag paper gummed with the best adhesive. A large series of labels may be outlined on a sheet of paper from which a permanent zinc block may be made. Any good job printer is able to furnish the label paper stock, have it gummed, and do the printing. Such sheets of labels are handily stored, easily cut, and suitable to write on with permanent India ink.

To apply labels to slides. Labels should be applied one at each end of the slide. They may be handled by forceps, dipping a single one wholly in water and applying it directly to the slide. A clean blotter beneath the slide and another to press down the label and to absorb the excess moisture around the edges aids in the process. With only a small amount of care the labels are soon placed squarely and tightly to the slide. With the blank labels in place it is very easy to center the specimens when mounting. The right-hand label is for the name of the insect and the left-hand one is for the name of the host plant, the locality, date and name of collector. This label is permanent, whereas the right-hand label may be changed with the scientific name of the insect.

VII. Mounting—Finished Slide

One or more properly cleared specimens are placed in the medium on the slide and after each specimen is carefully spread, with all the appendages properly arranged, the cover slip is applied as nearly horizontally as possible and carefully directed over the

specimens. The slides are placed in flat trays for drying. Labeling may be done with a croquill pen and India ink.

After the medium is thoroughly dry—2 to 4 weeks—the overflow may be removed around the edges of the cover slip with a safety razor blade or scalpel and the slide is then ready for ringing.

(1). *Ringing.* Ringing is accomplished by the use of a ringing table available from scientific supply houses. A No. "0" sable hair brush is most desirable for applying the ringing compound.

(2). *Protecting the labels.* The written labels may be protected from wear and weathering by applying two coats of dammar or other varnish.

This treatment also fastens the labels permanently to the slides and prevents wear, tear, and smearing. Old labels that have begun to darken and decay may be saved by varnishing.

References

DOETSCHMAN, W. H. 1944. Some suggestions in microtechnique particularly useful in microentomology and parasitology. Trans. Am. Micros. Soc., 63(2):175-178.

ELTRINGHAM, H. 1930. Histological methods for entomologists. Clarendon Press, Oxford, pp. 96-104.

GATER, B. A. R. 1929. An improved method of mounting mosquito larvae. Bul. Ent. Res., 19:367-368.

GROAT, R. A. 1939. Two new mounting media superior to Canada balsam and gum dammar. Anatomical Rec., 74:1-6.

HERSHBERGER, RUTH V. 1946. Differential stains for insect tissues. Ohio Jour. Sci., 46:152-162.

IMMS, A. D. 1929. Some methods of technique applicable to entomology. Bul. Ent. Res., 20:165-171.

JONES, BRYN. 1946. Impregnating polyvinal alcohol with picric acid for the simultaneous staining and permanent mounting of Acarina. Proc. Royal Ent. Soc. London, 21, pts. 10-12:85-86.

LEE, A. BOLLES. 1921. Microtomists' Vade-Mecum. Ed. 10, Blakiston, Philadelphia.

NYE., W. P. 1947. A simple method of mounting aphids. Pan-Pac. Ent., 23:73-74.

SMART, J. 1943. Mounting microscope slides. *In* Insects of Medical Importance. British Mus. Nat. Hist., pp. 263-265.

SWAN, D. C. 1936. Berlese's fluid: remarks upon its preparation and use as a mounting medium. Bul. Ent. Res., 27:389-391.

THORNE, GERALD. 1935. A new slide-ringing material. Proc. Helminthological Soc., 2:98.